Video Article

Retrieval of Mouse Oocytes

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Abstract

To date, only a few studies have reported successful manipulations of Peromyscus embryogenesis or reproductive biology. Together with the Peromyscus Genetic Stock Center (http://stkctr.biol.sc.edu), we are characterizing the salient differences needed to develop this system. A primary goal has been to optimize oocyte/early embryo retrieval.

Protocol

Embryo/Oocyte retrieval

1. At least two hours prior to retrieval of embryos/oocytes, KSOM is placed in the 37ºC CO_2 incubator and FHM must be thawed from -20°. A glass dish containing three concave shallow wells is used to house drops of KSOM in the incubator. To prevent dehydration, the KSOM and dish are placed in a lidded container that has water at the bottom. This apparatus prevents evaporation without the use of mineral oil.
2. The oviducts from an ovulating female are placed in FHM media. To ensure orientation and an intact infindibulum, a small section of the ovary is cut along with the oviduct and small section of the uterus. A blunt 30 gauge needle is used to expel embryos/oocytes from the oviduct by inserting the blunt needle into the infindibulum. The infindibulum must be used to retrieve the embryos since the oviduct tubing is too small for a needle to pass. In the event that the infindibulum is destroyed, the oviduct must be either sliced open or embryos squeezed out.
3. Embryos/oocytes are found in the FHM media by focusing the microscope on the fat droplets which have sunk to the bottom of the Petri dish. Embryo/oocyte isolation can be achieved at 20x as this provides a larger viewing area than high magnification. Embryo/oocytes look like round fat droplets that have a clear ring around it. Mouth pipetting, using a pulled glass capillary, is used to move embryos/oocytes to KSOM for incubation and further manipulation.

Procedure for Oocyte Retrieval

Preparation

1. Thaw FHM and prepare kSOM.
2. A few hours earlier, place kSOM in egg retrieval container and place in tip box with water in the bottom, place in CO_2 incubator.
3. Bring cages to the lab and kill the harvesting females.
4. Cut out the oviduct being careful to cut some of the ovary and some of the uterus to ensure that you do not cut off part of oviduct.
5. Place in FHM media in small Petri dish.
6. Pour a couple more FHM media Petri dishes to be used for flushing.

Flushing

1. Use a flushing needle (30 gauge blunt) connected to a 1ml needle with spiral end.
2. Take one oviduct at a time and using needle, insert into infundibulum found near the part of ovary left attached. If the infundibulum is destroyed and cannot be used, then take two forceps and hold one end of oviduct. With the other forcep, gently squeeze, like a toothpaste tube, to eject any embryo's.
   Note: This will create a large amount of mess in the Petri dish, as a lot of tissue will come with it, so you may want to use a different Petri dish afterwards.
3. To see embryos, focus microscope on fat pieces and look for round "fat piece" with clear circle around it.
   Note: The embryos have the same look as the fat, so it may be hard to find. The embryos sink to the bottom, so look there.
4. Do vaginal smears of recipient females. At this stage, they should be pseudopregnant.

Discussion

In this article, we demonstrate the retrieval of oocytes from ovulation-induced (superovulated) Peromyscus. The use of superovulation (as opposed to natural estrous cycle) is common due to the much greater resulting numbers. However, caution must be taken in interpreting results utilizing such oocytes/embryos, as both the hormones used to induce ovulation and culturing may have effects.

When estrous has been confirmed, females are euthenized and oviducts recovered along with a small portion of ovary. The small region of ovary is used as a marker for the location of the infindibulum. The infindibulum sits next to the ovary, often times projecting through a thin layer of tissue. On the other side of this tissue, the rest of the oviduct is coiled and eventually leads into the vagina. It is best to push fluid through the infindibulum to extract the oocytes. This direction is necessary due to a one way valve located at the vagina-oviduct interface. The oocytes may be harmed if they are projected backwards through the valve.

A 30-gaugle blunt end needle is inserted into the infindibulum. The blunt end needle will fit the infindibulum opening, but will not fit into the oviduct tubing. Therefore, care must be taken to ensure the infindibulum is not damaged during dissection or oocyte retrieval. If the infindibulum is damaged, the oviduct tubing can be squeezed via forceps to allow any trapped oocytes to exit.

Assuming the female was in estrous, the reproductive organs will have a red appearance due to increased blood supply and appear swollen. We observed differences in the two deer mouse species tested: P. polionotus had much fattier ovaries and oviducts than P. maniculatus, making oocyte retrieval more difficult.

Once the oocytes have removed from the oviduct, they may be observed by light microscopy at low magnification. Viewing the oocytes at a lower magnification allows the user to scan the Petri dish quickly. The microscope should be focused on the bottom layer of fat granules. The oocytes will look like perfectly round fat granules with a clear ring around the outside. To aid in detecting the oocytes, try adjusting the light projecting through the Petri dish. In addition, if the Petri dish is lightly shaken oocytes will not move compared to the fat droplets. These hints may help the novice researcher to locate the oocytes; however, it is likely that several trials will be necessary before one is comfortable with the procedure.

The isolated oocytes may be used in a variety of experiments including production of stem cell lines, production of chimeras, or genetic manipulations. While we have found considerable variation in the parameters and timing required to induce ovulation, the general techniques for retrieval should be applicable to many rodent species (deer mice are ~ 30 million years diverged from both laboratory mice and rats). We are particularly hopeful that this will benefit those working in novel systems where such techniques are not well-established.

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References

